GENERAL INSTRUCTIONS FOR TREATNENT OF EARLY-STAGE
SUBCUTANEOUS XENOGRAFTS (R2 PROTOCOL)

ANIMALS:

Propagation: Athymic random bred (NCr-nu) mice.

Testing: Athymic random bred (NCr-nu) mice.

Weight: Mice should have a minimum weight of 18 g for males and 17 g for females.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted in comments.

EXPERIMENT SIZE:

General Testing: **6-10** animals per test group.

Control Group: A minimum of 20 control animals must be used.

TUMOR TRANSFER FOR PROPAGATION AND TESTING:

Suspension: Prepare a suspension of diluted ascitic fluid so that a 0.1ml portion contains 1 x 10(6) cells.

Fragment: Prepare a 30-mg (acceptable range 20-40 mg) fragment from 300-1000 mg sc donor tumor without ulcerations.

Site: Implant sc either 0.1 ml of suspension containing 1×10^{6} cells or a 30-mg fragment into axillary region with puncture in inguinal region. Implant sufficient animals so that tumors may be selected in the proper size range.

Tumor Stage: When the median tumor size reaches 80-160 mg (the range of individual tumor sizes should be within 63-200 mg).

TEST REQUIREMENTS:

Staging Day: Measure the tumor dimensions to the nearest 1.0 mm. Record tumor measurements (mm) and animal weights (g) for individual mice on staging day (SD). Randomize the selected mice (bearing tumors in the specified range) into the appropriate experimental groups and individually identify mice as appropriate.

Deaths: Record deaths daily.

Animal Weights and
Tumor Measurements:

Weigh animals and measure tumors twice **weekly.**

Treatments:	Administer test agent based on the individual body weights on the specified days of treatment by the specified treatment route (see attached table for general treatment schedule).
Toxicity:	Record individual animal weights on the tumor measurement days. If mice have a 20% or greater loss in body weight not associated with tumor growth, the dose should be considered toxic.
Early Sacrifice:	If individual tumors approach 5 g or more, the mice should be sacrificed and tumor dimensions and animal weights recorded.
Evaluation Day:	At least 14 days after the last treatment, unless tumor growth requires earlier sacrifice, end and evaluate experiment. Record individual tumor measurements and animal weights.

EVALUATION OF ACTIVITY:

The following parameters will be recorded/calculated:

- number of tumor-free animals
- number of drug-related deaths
- number of no takes
- optimal T/C %
- % T-C/C
- median days to achieve a defined tumor weight -net log cell kill

REFERENCE DATA FOR SCHEDULING TREATMENT OF SC HUMAN TUMOR XENOGRAFTS

The treatment schedules currently in use were described in Instruction 420, issued August 27, 1993. A new table of reference data and treatment schedules has been prepared to include new tumor lines, updated data and requested changes in the schedules (eliminating weekly schedules). The doubling time of the tumors at 200 to 400 days was used to establish the schedule as in the previous instruction. The data in the table includes results of experiments reported by Julyl5,1995, as well as older data for tumors that have not been used during the past year. Additional, and in some instances more reliable data, indicated that the schedule should be modified for several tumor lines.

Under Days to Reach 80 to 160 mg, the mean of the staging days for the five most recent R2 protocol studies and the range of these staging days, when available, was used. When fewer than **five** studies were available, two to four sets of data were used or single values were included and indicated. When the tumors were staged at a larger size, or infrequent tumor measurements were made, the ranges were obtained by extrapolation of the available growth data but means are not shown. The doubling times were obtained from the same studies used for the staging data and, in some of the older data, the doubling times were obtained from extrapolations of the growth data.

The doubling times, the calculated growth in the interval between first and last treatment and the related treatment schedules are as follows:

1.2 to 2.5 day DT (1.0 to 0.48 log(10) growth in 4 days)	qd x 5 (SD)
2.6 or longer DT (0.93 log(10) or less growth in 8 days)	q4d x 3 (SD)

Tumor measurements 2 x wk are recommended for the initial evaluation of compounds. The schedule for tumor measurement in studies for further evaluation as follows: those tumors with DT of 1.2 to 2.5 days may be measured 3 x wk to limit the maximum calculated growth to 0.6 log(10) or less between measurements. The growth response to treatment during the rapid growth phase is important in evaluation of the agent. Tumor measurements 1 x wk beginning two weeks after staging are adequate for tumors with a mean DT that exceeds 7 days.

In Vivo Characterization and Reference Data for SC Human Tumor Xenografts (August 16, 1995)

			Days to Reach 80 to 160 mg		DT at 200-400 mg		Treatment
	Code	Tumor Line	Mean	Range	Mean	Range	Schedule
COLON							
	YG	COLO 205	13	12-14	3.5	2.6-5.7	q4d x 3 (SD)
	YO	COL0 32ODM	N.A.	8-14	5.3	4.1-6.2	q4d x 3 (SD)
		COLO 741	N.A.	>12	9.5(1)		q4d x 3 (SD)
	JP	DLD-1	9	8-13	3.4	2.1-5.6	q4d x 3 (SD)
	QI	HCC-2998	16	13-21	4.5	2.7-6.3	q4d x 3 (SD)
	QE	HCT-15	6	6-7	4.2	3.1-5.6	qd x 5 (SD)
	YK	HCT-116	12	9-15	2.5	1.2-4.8	qd x 5 (SD)
	C2	HT29	12	10-14	5.7	4.4-7.0	q4d x 3 (SD)
	YV	KM12	9	7-12	2.3	1.9-2.9	qd x 5 (SD)
	YU	KM12yR	N.A.	10-14	2.5	2.3-2.7	qd x 5 (SD)
	YQ	KM12L4a	N.A.	7-10	2.0	1.8-2.3	qd x 5 (SD)
	TA	KM2OL2	9	8-10	4.3	3.7-4.7	q4d x 3 (SD)
	JM	LOVO	N.A.	8-12	6.9	4.6-8.9	q4d x 3 (SD)
	QD	LS180	N.A.	8-11	2.3	1.9-2.6	qd x 5 (SD)
	QJ	NCI-H498	N.A.	11-14	5.9	4.4-6.7	q4d x 3 (SD)
		MLI-045	N.A.	17-22	3.3(1)		q4d x 3 (SD)
	JO	SW-620	8	6-8	2.1	15-2.5	qd x 5 (SD)
		UABC02	N.A.	13-IS	4.6	3.7-5.2	q4d x 3 (SD)
		UABOV1	N.A.	20-30	7.2	6.6-7.5	q4d x 3 (SD)
CNS							
	QK	SF-295	7	6-8	1.4	1.2-1.7	qd x 5 (SD)
	YM	SNB-19	N.A.	10-20	6.4	3.1-9.4	q4d x 3 (SD)
	YR	SNB-7	N.A.	7-11	2.7	2.4-3.1	q4d x 3 (SD)
	BG	SNB-75	10	9-13	2.7	1.8-3.1	q4d x 3 (SD)
		SW-608	N.A.	6-12	2.5	2.4-2.7	q4d x 3 (SD)
	TE	TE671	N.A-	10-15	3.1	2.5-3.7	q4d x 3 (SD)
	UG	U251	12	7-14	5.8	2.9-8.9	q4d x 3 (SD)
		U-87 MG	N.A.	6-9	2.1(1)		qd x 5 (SD)
	OL	XF 498	N.A.	13-15	4.4	2.6-5.6	q4d x 3 (SD)
LEUKEMIA/LYMPHOMA							
	BE	CCRF-CEM	N.A.	14-24	4.5	4.3-4.6	q4d x 3 (SD)
	HL	HL-60(TB)	11	9-13	2.7	2.2-3.3	q4d x 3 (SD)
	BD	MOLT-4	N.A.	12-20	3.2	2.0-4.0	q4d x 3 (SD)

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			Days to Reach 80 to 160 mg		DT at 200-400 mg		Treatment
	Code	Tumor Line	Mean	Range	Mean	Range	Schedule
LUNG							
		A427	N.A.	14-18	2.8	2.4-3.3	q4d x 3 (SD)
	LN	A549	N.A.	15-20	8.7	5.8-10.9	q4d x 3 (SD)
		CALU-6	N.A.	12-16	4.4	3.3-5.8	q4d x 3 (SD)
	TB	DMS 273	N.A-	7-12	1.7	1.6-1.9	qd x 5 (SD)
	JR	DMS114	N.A-	15-20	3.9	3.6-4.6	q4d x 3 (SD)
	QS	EKVX	N.A.	16-20	5.0	3.6-7.1	q4d x 3 (SD)
		HOP-27	N.A.	< 10	1.7(1)		qd x 5 (SD)
	BB	HOP-92	N.A.	20-25	9.3	6.9-12.5	q4d x 3 (SD)
	JD	NCI-Hl25M	N.A.	10-20	5.1	5.0-5.4	q4d x 3 (SD)
	JA	NCI-H23	14	10-20	3.3	2.0-4.6	q4d x 3 (SD)
	JH	NCI-H69	N.A.	10-15	4.41	4.2-4.5	q4d x 3 (SD)
	JK	NCI-H82	N.A.	12-16	4.21	3.7-4.6	q4d x 3 (SD)
	LV	NCI-H322M	15(1)		2.7		q4d x 3 (SD)
	CL	NCI-H460	7	6-8	1.8	1.1-2.5	qd x 5 (SD)
	JG	NCI-H520	N.A.	20-27	4.41	3.8-5.2	q4d x 3 (SD)
	JC	NCI-H522	13	10-16	3.5	2.2-5.9	q4d x 3 (SD)
		SHP-77	N.A.	18-24	4.1	3.6-4.3	q4d x 3 (SD)
	JZ	SK-MES-1	N.A.	12-16	3.3	2.8-3.9	q4d x 3 (SD)
MAMMARY							
		LXFL625	N.A.	14-18	4.2	3.0-5.3	q4d x 3 (SD)
		MAMGI-101	N.A.	30-40	13.4	12.0-15.9	q4d x 3 (SD)
	MZ	MAXF 401	N.A-	16-27	6.4	5.8-7.2	q4d x 3 (SD)
		MAXF 583	N.A.	35-40	10	7.3-11.9	q4d x 3 (SD)
	KC	MCF7	12	9-15	5.5	3.5-8.0	q4d x 3 (SD)
	KM	MDA-MB-231	17	13-27	4.7	2.8-9.3	q4d x 3 (SD)
	MK	MDA-MB-435	16	13-17	6.9	4.4-9.7	q4d x 3 (SD)
	MJ	MDA-N	13	9-15	9.1	6.9-12.4	q4d x 3 (SD)
	MB	MX-1	8	7-9	2.7	2.2-3.0	q4d x 3 (SD)
	MN	UISO-BCA-1	N.A.	12-18	4.1	2.8-5.4	q4d x 3 (SD)
	MO	ZR-75-1	6	5-7	1.7	1.4-1.9	qd x 5 (SD)
MELANOMA							
	CC	COLO 746	N.A-	15-25	7.9	5.2-12.7	q4d x 3 (SD)
		G298L	N.A.	12-15	4.9	4.7-5.0	q4d x 3 (SD)
	LO	LOX-IMVI	7	5-7	1.2	1.1-1.4	qd x 5 (SD)

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			Days to Reach 80 to 160 mg		DT at 200-400 mg		Treatment
	Code	Tumor Line	Mean	Range	Mean	Range	Schedule
	QA	M14	17	15-1	7.0	4.9-9.0	q4d x 3 (SD)
	QM	M19-MEL	N.A.	ca. 50	15.9	13.1-17.7	q4d x 3 (SD)
	YF	MALME-3M	N.A.	12-15	10.3	7.1-16.9	q4d x 3 (SD)
	QQ	SK-MEL-2	N.A.	20-30	5.3	4.8-6.1	q4d x 3 (SD)
	YP	SK-MEL-28	8	8-9	1.6	1.3-1.9	qd x 5 (SD)
	QC	SK-MEL-31	N.A.	20-30	5.7	5.2-6.1	q4d x 3 (SD)
	JQ	SK-MEL-5	14(1)		8.2(1)		q4d x 3 (SD)
	QN	UACC-62	13	12-14	2.8	2.7-3.0	q4d x 3 (SD)
	QP	UACC-257	N.A.	17-24	8.6(1)		q4d x 3 (SD)
	QF	UCSD 242L	N.A.	12-18	4.5	4.1-5.2	q4d x 3 (SD)
	QG	UCSD 354L	N.A.	8-12	3.8	2.5-4.5	q4d x 3 (SD)
		UCSD 462L	N.A.	16-20	6.5	6.1-7.2	q4d x 3 (SD)
	QH	UCSD 535L	N.A.	15-20	5.8	2.3-7.4	q4d x 3 (SD)
OVARIAN							
	YW	A2780	N.A.	7-10	2.3	1.7-3.1	qd x 5 (SD)
	YH	IGROV1	N.A.	13-24	5.5	4.2-6.6	q4d x 3 (SD)
	OC	OVCAR-3	7(1)		4.4(1)		q4d x 3 (SD)
	JX	OVCAR-5	15	14-23	3.4	2.2-4.3	q4d x 3 (SD)
	JY	OVCAR-8		20-30	12.3	12.2-12.5	q4d x 3 (SD)
	QR	SK-OV-3	15	13-19	3.2	2.8-3.6	q4d x 3 (SD)
PROSTATE							
	JV	DU-145	15	13-16	3.2	1.4-5.9	q4d x 3 (SD)
	KL	JCA-1	10	8-14	4.3	1.3-5.9	q4d x 3 (SD)
	ZA	LNCAP	8	7-10	2.1	1.6-2.7	qd x 5 (SD)
	KH	PC-3	11	7-13	2.2	1.3-3.2	qd x 5 (SD)
	KE	PC-3-M	11	8-14	2.5	2.1-3.2	qd x 5 (SD)
RENAL							
	RH	786-0	13(1)		6.5(1)		q4d x 3 (SD)
	YJ	A498	15	12-16	3.3	2.6-4.4	q4d x 3 (SD)
		A704	N.A.	13-28	6.5	5.2-7.6	q4d x 3 (SD)
	YE	CAKI-1	13	12-15	2.2	1.3-3.4	qd x 5 (SD)
	RF	RXF 393	7	5-9	3.2	2.3-4.0	q4d x 3 (SD)
	RG	RXF 631	10	9-10	2.2	1.2-3.2	qd x 5 (SD)
	JW	SN12Kl	N.A.	8-14	2.3	2.0-2.5	qd x 5 (SD)
	YS	SN12Al	N.A.	10-15	5.5	4.1-6.8	q4d x 3 (SD)

In Vivo Characterization and Reference Data for SC Human Tumor Xenografts (August 16, 1995)

		Days to Reach 80 to 160 mg		DT at 200-400 mg		Treatment
Code	Tumor Line	Mean	Range	Mean	Range	Schedule
YL	SN12C	12	8-15	4.9	3.6-5.8	q4d x 3 (SD)
YT	SN12LI	N.A.	12-18	7.1	5.9-9.6	q4d x 3 (SD)
QB	SN12SI	N.A.	10-20	8.1	6.9-9.3	q4d x 3 (SD)
MISCELLANEOUS						
	BZRT-3		10-14	4.1	3.1-4.6	q4d x 3 (SD)
YN	MHM-8 Sarcoma		7-15	1.9(1)	1.7-3.3	qd1 x 5 (SD)

Calculated from median tumor weights determined on staging and evaluation days or on multiple days as available. Data were not available to calculate DT from 200 to 400 mg for individual tumors. Single values are indicated by (1) following the number. N.A. (not available) is shown in the column for Mean Days to Reach 80 to 160 mg when tumor measurements were not obtained on tumors in that size range. The ranges were obtained from extrapolation of the growth data.

Note: To accommodate the narrow weight range for staged tumor size, 50% - 75% excess mice may have to be implanted with tumor fragments.

Unless otherwise specified, activity is calculated by the A method.

Host body weights are recorded on staging day (Weigh Day 1) and on tumor measurement days during treatment and for two weeks after the end of treatment. Weigh weekly thereafter.

Tumor measurements are made 2 x wk for initial evaluation of agents and must be made on the day of animal weights because the computer program calculates net animal weight.